

**PATENT**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of:

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William Wu

Serial No.: Unknown

Filed: Concurrently Herewith

For: METHODS AND REAGENTS FOR  
INACTIVATING RIBONUCLEASES

Group Art Unit: Unknown

Prior Examiner: Unknown

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**PRELIMINARY AMENDMENT**

Commissioner of Patents  
Washington, D.C. 20231

Sir:

Applicant respectfully submits this Preliminary Amendment in the above-referenced case.

Consideration of this case in view of the amendment made herein is respectfully requested.

**AMENDMENT**

**In the Specification:**

Please amend the specification as follows:

At page 1, line 5, please insert the following paragraph:

--This application is a continuation of co-pending international application number PCT/US99/22230 filed 24 September 1999, which claims priority to U.S. application serial number 09/160,284 filed 24 September 1998.--

**In the Claims:**

Please cancel claims 1-53, without prejudice or disclaimer.

Please add the following new claims:

--54. A method comprising:

- (a) obtaining at least one cell;
- (b) obtaining a reducing agent;
- (c) preparing an admixture of the cell and the reducing agent; and
- (d) heating the admixture.

55. The method of claim 54, wherein preparing an admixture of the cell and the reducing agent is further defined as comprising preparing an extract of the cell and preparing an admixture of the extract of the cell and the reducing agent.

56. The method of claim 55, wherein preparing an admixture of the extract of the cell and the reducing agent comprises:

- (a) first preparing the extract; and
- (b) then mixing the extract with the reducing agent.

57. The method of claim 55, wherein preparing an admixture of the extract of the cell and the reducing agent comprises:

- (a) first mixing the cell and the reducing agent; and
- (b) then preparing the extract from the cell in the presence of the reducing agent.

58. The method of claim 54, further defined as a method for producing cDNA from one or more cells and further comprising incubating the admixture with reverse transcriptase under conditions to allow reverse transcription.

59. The method of claim 58, further comprising amplifying the products of the reverse transcription.

60. The method of claim 58, further comprising incubating said admixture with a deoxyribonuclease prior to the reverse transcription reaction.

61. The method of claim 54, wherein the said reducing agent is DTT,  $\beta$ -mercaptoethanol, cysteine, or dithioerythritol.

62. The method of claim 54, wherein the reducing agent is DTT.

63. The method of claim 54, wherein said the final concentration of the DTT is between 1 and 200 mM in the admixture.

64. The method of claim 63, wherein the final concentration of DTT is 20 mM in the admixture.

65. The method of claim 54, wherein said reducing agent is  $\beta$ -mercaptoethanol.

66. The method of claim 65, wherein the final concentration of  $\beta$ -mercaptoethanol is between 1 and 200 mM in the admixture.

67. The method of claim 54, wherein said reducing agent is cysteine.

68. The method of claim 67, wherein the final concentration of cysteine is between 1 and 200 mM in the admixture.

69. The method of claim 54, wherein the reducing agent is comprised in a buffer composition prior to admixing.

70. The method of claim 54, wherein the admixture is heated to at least 37°C.

71. The method of claim 70, wherein the admixture is heated to at least 60°C.

72. The method of claim 54, wherein the admixture is heated for at least 4 minutes.

73. A method for producing cDNA from one or more cells comprising:

- (a) obtaining at least one cell;
- (b) obtaining a reducing agent;
- (c) preparing an admixture of the cell and the reducing agent;
- (d) heating the admixture; and
- (e) incubating the admixture with reverse transcriptase under conditions to allow reverse transcription.

74. The method of claim 73, wherein preparing an admixture of the cell and the reducing agent is further defined as comprising preparing an extract of the cell and preparing an admixture of the extract of the cell and the reducing agent.

75. The method of claim 74, wherein preparing an admixture of the extract of the cell and the reducing agent comprises:

- (a) first preparing the extract; and
- (b) then mixing the extract with the reducing agent.

76. The method of claim 74, wherein preparing an admixture of the extract of the cell and the reducing agent comprises:

- (a) first mixing the cell and the reducing agent; and
- (b) then preparing the extract from the cell in the presence of the reducing agent.

77. The method of claim 73, further comprising amplifying the products of the reverse transcription.

78. The method of claim 73, further comprising incubating said admixture with a deoxyribonuclease prior to the reverse transcription reaction.

79. The method of claim 73, wherein the said reducing agent is DTT,  $\beta$ -mercaptoethanol, cysteine, or dithioerythritol.

80. A kit for producing cDNA from a cell, comprising, in a suitable container:

- (a) a buffer; and
- (b) a reducing agent.

81. The kit of claim 80, wherein the buffer and the reducing agent are comprised in the same container.

82. The kit of claim 80, further comprising, in one or more container(s):

- (c) a reverse transcription buffer
- (d) a reverse transcriptase; and
- (e) a dNTP mix.

83. The kit of claim 80, further comprising a deoxyribonuclease.

84. The kit of claim 80, wherein said reducing agent is DTT.

85. The kit of claim 80, further comprising an RNase inhibitor.
86. The kit of claim 80, further comprising Armored RNA®.
87. A kit for producing cDNA from a cell comprising, in one or more suitable container(s):
- (a) a cell lysis buffer;
  - (b) a deoxyribonuclease;
  - (c) an RNase inhibitor;
  - (d) a reverse transcription buffer;
  - (e) reverse transcriptase;
  - (f) dNTPs; and
  - (g) Armored RNA®.--

#### REMARKS

The specification has been amended to recite the priority data, to cancel claims 1-53 of the prior application, and to add new claims 54-87.

The filing fee has been calculated after amendment of the claims by the preliminary amendment. Should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required, the Assistant Commissioner is hereby authorized to deduct said fees from Fulbright & Jaworski Deposit Account No. 50-1212/10102270/MBW.

Respectfully submitted,



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